O-1

Hypoxia ameliorates the wound healing ability of extracellular vesicle (EVs) derived from adipose tissue-mesenchymal stem cells (AT-MSCs)

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Mesenchymal stem cells (MSCs) play the key role in wound healing process via the paracrine effects by secreting cytokines and small particles. Extracellular vesicles (EVs) are small particles containing of nucleic acids and proteins which can be transferred to the target cells. Recent studies reported MSC-derived EVs (MSC-EVs) promotes the wound healing; therefore EVs-based therapy becomes the attractive strategy in clinical application to avoid the immunorejection. Furthermore, it is reported that hypoxic treatment enhances the wound healing ability of MSCs. However, the detail mechanism how hypoxic induction affects EVs to improve the wound healing have not been elucidated yet. In the present study, we examined the effects of hypoxic induction on EVs by comparing the wound healing function of EVs derived from normoxic and hypoxic AT-MSCs. Results showed that hypoxic induction upregulated the expression of wound healing-related genes in EVs. Of note, hypoxic EVs showed higher ability to support the wound healing function of the target cells, including MSCs and endothelial progenitor cells (EPCs) by the enhancemet of the migration and wound healing related gene expression, including CXCR4, SDF1, bFGF and VEGF. Moreover, transplantation of hypoxic EVs into diabetic mice significantly reduced the wound area within 7 days compared to those transplanted with normoxic EVs. Hypoxic EVs showed significantly high ability to recruit the inflammatory cells and support the angiogenesis at the wound site. Therefore, hypoxic-treated EVs can be considered as a promising candidate for delayed wound healing in future.

O-2

The role of inhibitory immunoreceptor in sepsis model

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Sepsis is defined as life-threatening organ failure caused by a dysregulated host immune response to infection, resulting in around 5.3 million deaths worldwide every year. The inflammatory immune response during sepsis is initiated by the activation of Toll-like receptors (TLRs) and cytokine signaling. Although TLRs-mediated signaling may cause excessive amount of inflammatory mediators production that leads to multi-organ failure, it is also important for clearance of pathogens. Therefore, it is important for controlling sepsis to clarify the regulatory mechanisms of TLR-mediated signaling.

We have reported that an inhibitory immuno-receptor, which has immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in its cytoplasmic region and is expressed on mast cell (MC), macrophage/monocyte, dendritic cell and neutrophil, inhibits high-affinity receptor for IgE (FcεRI)- and TLR2-mediated signalings in MCs, resulting in suppression of passive systemic anaphylaxis and dermatitis, respectively. We next aimed to clarify the role of this receptor in sepsis.

Wild-type (WT) and inhibitory immunoreceptor-deficient mice (KO) were subjected to a well-defined cecal ligation and puncture (CLP)-induced polymicrobial peritonitis model that is closely related to the nature and course of clinical sepsis. The mortality rate of WT mice reached 100% at 72 h after CLP compared with around 60% in KO mice. Blood and peritoneal cavity bacterial colony counts at 2 h after CLP was lower in KO mice, suggesting that bacterial clearance was enhanced in KO mice. Correspondingly, neutrophils recruitment and the level of inflammatory cytokines in peritoneal lavage were increased in KO mice compared with WT mice at 2 h after CLP. *In vitro* analysis demonstrated that stimulation of bone marrow-derived cultured mast cells (BMMCs) with TLR4 ligand, LPS, induced higher inflammatory cytokines production in KO-derived BMMCs than that of WT mice, suggesting that this inhibitory immunoreceptor inhibits TLR4-mediated signaling in MCs.

Together, these results suggested that this inhibitory immunoreceptor suppressed inflammatory cytokines production and neutrophils recruitment after CLP through inhibition of TLR-mediated signaling, leading to accelerated sepsis.

O-3

Ablation of central serotonergic neurons decreased REM sleep and attenuated arousal response

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Sleep is a fundamental animal behavior observed widely across the animal kingdom, including worms, jerry fish, and vertebrates. However, the regulation of sleep/wakefulness by distinct groups of neurons remains largely unknown. Although monoaminergic neurons such as dopaminergic, noradrenergic, and histaminergic neurons are usually regarded as wake-promoting neurons, role of serotonergic neurons is inconclusive because of inconsistent results of previous studies. This inconsistency may be partly due to the depletion of peripheral serotonin, disturbed neural circuit development, and compensatory regulation in sleep/wakefulness. More than 90% of serotonin work in peripheral tissues such as adipocytes, liver, and pancreas. During brain development, serotonin modulate thalamocortical axon guidance related to sleep regulation. In addition, the mild sleep/wake phenotype in mice deficient in for tryptophan hydroxylase 2, the rate-limiting enzyme in the synthesis of serotonin showed mild sleep phenotype, might be due to compensations by other sleep/wake-regulating neurons.

Here, we performed selective ablation of central serotonergic neurons in newly developed *Rosa-diphtheria toxin receptor (DTR)-tdTomato* mouse line that was crossed with $Pet1^{cre/+}$ mice to examine the role of serotonergic neurons in the sleep/wake behavior of adult mice. Intracerebroventricular administration of diphtheria toxin completely ablated tdTomato-positive cells in $Pet1^{Cre/+}$*; Rosa-DTR-tdTomato* mice. Electroencephalogram/electromyogram-based sleep/wake analysis demonstrated that central 5-HT neuron ablation in adult mice decreased the time spent in rapid eye movement (REM) sleep, which was associated with fewer transitions from non-REM (NREM) sleep to REM sleep than in control mice. Central 5-HT neuron-ablated mice showed attenuated wake response to a novel environment and increased theta power during wakefulness compared to control mice. The current findings indicated that adult 5-HT neurons work to support wakefulness and increase REM sleep time through a biased transition from NREM sleep to REM sleep.

O-4

Regulation of clathrin-independent cargo trafficking by the ubiquitin-specific protease TRE17/USP6 and the small GTPase Arf6

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Plasma membrane proteins that enter cells by endocytosis are sorted either to lysosomes for degradation or recycled back to the plasma membrane, thereby cells regulate localization and expression levels of the proteins at the cell surface. The sorting process is regulated by cycles of ubiquitylation and deubiquitylation: cargo proteins tagged with ubiquitin are transported to and degraded in lysosomes, while deubiquitylation of cargo proteins targets them to recycling. We have previously demonstrated that TRE17/USP6, a member of the ubiquitin-specific proteases (USPs), promotes recycling of plasma membrane proteins which enter cells through clathrin-independent endocytosis (CIE) by deubiquitylating them. Through this process, TRE17 stabilizes and increases the cell surface levels of target CIE cargo proteins, including MHCI, CD44, and CD98. However, the molecular mechanism how TRE17 recognizes target proteins and regulate CIE cargo trafficking remains to be elucidated. Interestingly, it has been reported that TRE17 interacts with the small GTPase Arf6, which is involved in CIE endosomal membrane trafficking. In this study, we investigated the functional link between TRE17 and Arf6 in TRE17-regulated CIE cargo trafficking.

In HeLa cells, TRE17 localizes at the plasma membrane and tubular endosomes, where it co-localizes with CIE cargo proteins and Arf6. In contrast, the TRE17 mutant which cannot bind to Arf6 (TRE17 A6-) mislocalized and distributed to the cytoplasm, indicating that subcellular localization of TRE17 depends on binding to Arf6. Overexpression of TRE17 WT in HeLa cells suppressed the CIE cargo sorting to lysosomes and stabilized them, whereas overexpression of TRE17 A6- did not exhibit those phenotypes. In contrast, when TRE17 A6- is anchored to the plasma membrane and CIE endosomes by appending the C-terminal farnesylation signal of H-Ras to its C-terminus, TRE17 A6- then suppressed the CIE cargo sorting to lysosomes. Taken together, we propose a model that Arf6 recruits TRE17 to the compartment where TRE17 deubiquitylates CIE cargo proteins.

O-5(P-9)

Synthesis and functionalization of furo[2,3-*b*]pyridine core

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The furo[2,3-*b*]pyridine core has recently received extensive attention from the medicinal chemistry community as a useful pharmacophore for the development of biological active compounds, but despite their importance, synthetic methodologies for furopyridines remain limited. We develop a concise synthetic strategy to synthesize this core via the heterocyclization of pyridine-*N*-oxide derivatives. Mild, metal-free conditions were successfully applied to produce a range of 2-(alkyl or aryl)-3-ethylcarboxylate-furo[2,3-*b*]pyridines in yields of 50-91%. Then, the chemical reactivity of this heterocyclic framework was explored to develop straightforward methods for its functionalization. Considering that the progress on C–H activation had being focus on find alternative approaches to improve the existing methods to broaden their scope and functional group compatibility, the medicinal chemistry community realized that C–H functionalization methods offer an opportunity to explore and expand the chemical space more effectively rather than relying solely on conventional synthetic approaches, especially for underexplored scaffolds as the furo[2,3-*b*]pyridine. Therefore, we successfully explored the pyridine moiety reactivity by C–H amination, C–H iodination and borylation reactions, although C–H fluorination and radical C–H arylation processes were not as efficient. In addition, while the furopyridine core proved stable under basic conditions, the ring-opening reaction of the furan moiety with hydrazine generated a valuable new pyridine-dihydropyrazolone scaffold. Our library of furopyridines are under biological activity evaluation against *Mycobacterium tuberculosis*.

O-6

The Different Stress-sensing activities of Keap1a and Keap1b in Zebrafish

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The Keap1-Nrf2 pathway is known as an evolutionary conserved mechanism to protect cells from oxidative stress and xenobiotics. Keap1 is a repressor of Nrf2, mediating its ubiquitination and proteasomal degradation under basal conditions, while interestingly, can also sense a variety of stresses and chemicals with its reactive cysteines and then changes the protein conformation to disrupt the proper Keap1-Nrf2 interaction for the ubiquitination, leading to stabilization of Nrf2. Thus, Keap1 has been considered as "a stress sensor" for the Nrf2 activation. In zebrafish, there are two Keap1 co-orthologous, Keap1a and Keap1b, which we previously suggested to have different stress-sensing activities by gain-of-function analyses

In this study, we generated knockout zebrafish lines of both Keap1a and Keap1b by the CRISPR-Cas9 system to confirm the differences in stress-sensing activities between Keap1a and Keap1b by loss-of-function analyses. Using these lines, we analyzed induced expression of an Nrf2 target gene *gstp1* in response to a variety of Nrf2 activators, such as sulforaphane (SF), diethyl maleate (DEM) and15-deoxy-Δ12,14-prostaglandin J_2 (15d-PGJ_2) by whole-mount *in situ* hybridization. In Keap1a-knockout embryos, *gstp1* induction in response to SF and DEM was elevated compared with wild-type embryos, while that in response to 15d-PGJ_2 was reduced. On the other hand, Keap1b-knockout embryos showed opposite results: the response to 15d-PGJ_2 was upregulated and to SF and DEM was downregulated. These results suggest that Keap1a has higher affinities to 15d-PGJ_2 than Keap1b and, conversely, Keap1b interacts stronger with SF and DEM than Keap1a. The finding of the difference in stress-sensing specificity between Keap1a and Keap1b will give us a significant hint to identify sensor sites for these Nrf2 activating stressors.

O-7

Elucidation of the role of Nucleophosmin in Adenoviral genome packaging and capsid assembly

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Adenovirus (Ad), despite causing several human infections, is widely used as a gene vector. Its basic biology in terms of gene replication and transcription has been well studied, however the mechanism of adenoviral genome packaging and capsid assembly are yet to be fully elucidated. Elucidation of these processes could lead to the discovery of a novel DNA packaging mechanism of Ad, which could help in the understanding of the packaging mechanism of other dsDNA viruses. Therefore, this research could contribute to the development of an anti-adenoviral drug and to the improvement of gene vector design. Our laboratory identified Nucleophosmin (NPM1) as a host stimulatory protein of Ad DNA replication. Recent studies demonstrated that depletion of NPM1 decreased Ad virus production without affecting the Ad transcription and replication, suggesting its role in Ad genome packaging or capsid assembly. NPM1 has also been implicated in these life processes of other dsDNA viruses. Hence, the aim of this study is to elucidate the role of NPM1 in Ad life cycle.

To clarify its function, we investigated the cellular localization of NPM1 during Ad infection cycle. We demonstrated that the localization of NPM1 is not affected until viral genome replication starts. Upon initiation of DNA replication, NPM colocalized with viral replication protein, DBP. When viral replication proceeds and newly synthesized viral DNA increases, NPM1 accumulates to the central region of viral DNA replication sites. Finally, NPM1 colocalized with the viral capsid protein, pVI. These results support the idea that NPM1 is involved in the genome packaging or capsid assembly. Furthermore, we investigated the interaction between NPM1 and genome packaging and capsid proteins. So far, we found that NPM1 directly interacts with IVa2, a genome packaging protein. We are now trying to elucidate the molecular mechanism by which NPM1 regulates these two Ad life processes.

O-8

The role of an inhibitory immunoreceptor in colitis model

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Extensive studies have been demonstrating that the gut microbiota modulates the development and function of the immune system and the dysbiosis plays a role in the pathogenesis of inflammatory bowel diseases (IBD), including Crohn's disease and ulcerative colitis (UC), as well as extra-intestinal disorders, including allergy, autoimmunity, and obesity. However, the molecular mechanisms how immune system modulates the gut microbiota remains incompletely understood.

We have reported that an inhibitory immunoreceptor, which is highly expressed in the myeloid lineage cells population and has an intracellular immunoreceptor tyrosine-based inhibitory motifs (ITIMs), which can recruit SHP-1 phosphatase and further negatively regulate Toll-like receptor-2 (TLR-2) signaling pathway. TLRs are the pattern recognition receptors that can detect a broad range of molecules of the pathogens including commensal bacteria, and a loss of TLRs signaling increases the susceptibility of colitis. Therefore, we aimed to clarify the role of this receptor in colitis model.

We treated wild-type (WT) and inhibitory immunoreceptor-deficient (KO) mice with dextran sodium sulfate (DSS) containing drinking water, which induces murine model of UC. The results from average body weight loss and their colon length demonstrated that KO mice showed more severe phenotype than WT mice. However, this phenotype was canceled upon cohousing WT and KO group, suggesting that KO mice may develop dysbiosis. Recent study reported that dysbiosis causes intestinal barrier dysfunction, we analyzed the expression of intestinal tight junction molecule, occluding and found that it was decreased in KO mice compared with WT mice, even in the naïve state. In addition, oral administration of FITC-labelled Dextran demonstrated that fluorescent detection of FITC in serum was increased in KO mice compared with WT mice, indicating that intestinal permeability was increased in KO mice in the naive state. Moreover, macrophage-specific conditional KO mice showed similar phenotype with systemic KO mice. In summary, the deficiency of this inhibitory immunoreceptor in intestinal myeloid cells population may modulate microbiota composition and further increase colitis susceptibility in mice. Therefore, this receptor may play an important role in intestinal barrier homeostasis through the regulation of the commensal bacteria composition.

O-9

Family Function perceived by Primary Caregivers raising a Child with Severe Motor and Intellectual Disabilities at home in Japan

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[Background & Aim]

The number of children with severe motor and intellectual disabilities (SMID) is recently increasing in Japan, and approximately 70 % of them are living at home. Support for adjusting their family function is one of the important roles of nurses working for these families, and it is said that there are some group characteristics. It is said that nurses should consider these features when they are planning and providing care for these families with special needs. Therefore our aim of this study is to clarify the actual situation and the group characteristics of family function perceived by primary caregivers raising the child with SMID at home in Japan.

[Methods]

We conducted the questionnaire survey for family members raising a child with severe motor and intellectual disabilities by mail to 212 special schools for physically handicapped children in all prefectures in Japan. The questionnaires were composed of Family Adaptability and Cohesion Evaluation Scale at Kwansei Gakuin (FACESKG-Ⅳ), and their family attributions and health related outcomes. Descriptive analyses and Kruskal-Wallis test were conducted. The significance level was set at 5 %.

[Results]

1233 primary caregivers responded our questionnaires, and 93.2% of them were mothers of the child with SMID. In regard to cohesion of family function, it showed these distribution; “rigid” 24.09%, “structured” 23.11%, “flexible” 47.53%, “chaotic” 5.27%. As to adaptability, “disengaged” 7.89%, “separated” 5.35%, “connected” 21.41%, “enmeshed” 65.37%. Family factors significantly related to both of cohesion and adaptability were the following factors; the duration of home care, caregiver burden, family empowerment, psychological health, the number of supporters for raising their child with SMID ($p=0.001 \sim 0.044$).

[Conclusions]

For the future, it would be better that nurses provide quality care for them with assessing their each family function on the basis of these results.

O-10

Label-free Raman imaging of elastic fibers and collagens in disease and healthy mouse aortic tissues

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Elastic fibers represent major components of the extracellular matrix (ECM). They are composed of elastin, fibrillin-1, and fibulin family proteins. Fibulin-4 and fibulin-5 bind to elastin and are essential for elastogenesis in vivo. Fibulin-5 knockout mouse (F5KO) shows elongation and tortuosity of the aorta. By deleting fibulin-4 specifically in vascular smooth muscle cells (SMKO), a disease model of ascending aortic aneurysm has been generated.

In this study, we imaged aortic tissues from F5KO, SMKO and wild-type (WT) mice by using Raman microscopy. The thoracic aortas were separated between ascending and descending segments and cross sections were prepared using cryopreserved samples. For each section, large-area Raman images and high-resolution Raman images were generated by using a 532 nm laser excitation. Principle component analysis (PCA) and true component analysis (TCA) were utilized to analyze the image data.

Raman imaging illuminated major four biochemical components in the tissues: (1) cells/nucleic acids, (2) collagen fibers, (3) elastic fibers, and (4) lipids. Large-area imaging data showed an overview of the ECM network. High-resolution data illustrated the location of each component in detail. Single Raman spectra were specifically generated from elastic fibers within the tissues. We identified differences in the Raman signature of elastic fibers between WT and mutant mouse aortic tissues.

Raman spectra were able to detect differences between healthy and diseased elastic fiber networks within aortic tissues. It might be a future technology for the label-free diagnosis of cardiovascular disease. High-resolution Raman imaging can shed light on the details of ECM architecture in diseased tissues.

O-11(P-26)

Understanding the transcriptional regulation of endoreduplication for tomato fruit growth regulation

Endoreduplication, during which cells increase their DNA content through successive rounds of full genome replication without cell division, is a widespread phenomenon and the major source of endopolyploidy in higher plants. In tomato fruits, the pericarp is composed of a heterogeneous population of cells that display high ploidy levels that often correlated with large cells. Endoreduplication is thought to play a key role in the rapid growth of cells. Yet, only few is known about the molecular mechanisms regulating the onset and progression of endoreduplication. Our objective is to reveal the transcriptional regulation of endoreduplication progression in relation to cell size control with the goal of understanding how a fruit regulates its overall cell volume, and thus its final size.

To explain why a heterogeneous population of cells co-exists within the pericarp and as a basis to dissect the genetic regulation of endoreduplication, we will determine the ploidy and cell size distribution in the pericarp of growing Tomato fruits and overlap it with ploidy-specific transcriptional information obtained from this tissue during development. The ploidy levels will be determined in situ by using a CRISPR/Cas9 based technology, CRISPR imaging, that makes use of a fluorescently tagged inactivated CAS9 addressed to a specific genomic locus by a Single guide RNA to visualize it.

To obtain a genome-wide ploidy-specific transcriptional information from fruit pericarp during development, nuclei from pericarp of fruits harvested at different growth stages were sorted based on their ploidy levels and used for RNA sequencing. By studying *in situ* the evolution of ploidy levels and cell areas in the fruit pericarp during development, spatial and temporal distribution maps of ploidy will be obtained for this tissue. The genome-wide ploidy-specific transcriptional analysis from the fruit pericarp will provide insights into the molecular pathways associated with endoreduplication progression.

O-12

Isolation of a pure population of differentiated cells using auto-erasable vector

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We have known that heterogeneity in differentiated cells is a huge problem in regenerative medicine. For example, if these heterogeneous cells are used for transplantation, it could lead to cancer formation. Thus, we would like to solve this problem and generate homogeneous differentiated cells.

In this research, we plan to develop an auto-erasable vector system, derived from the Sendai virus (SeV) vector, which allows efficient isolation of a pure population of differentiated cells. The auto-erasable vector was designed to harbor a differentiated cell specific miRNA target sequences, neomycin resistance gene as well as cytochrome P450 gene. After infection of the vector to mouse embryonic stem cells (mESCs), vector-positive cells will be selected by neomycin treatment. After differentiation of the mESCs, the cells express the specific miRNA, which helps eliminate the vector from the differentiated cells. Undifferentiated cells, which contain the virus expressing P450, will be removed by cyclophosphamide (CPA) treatment. These two steps of selection, in principle, should generate a pure population of differentiated cells that are free of the vector.

To establish such a system, we differentiated mESCs into neural stem cells (NSCs) as a model. Therefore, we chose two miRNAs which are expressed in NSCs. RT-qPCR of differentiating cells confirmed that one of the miRNAs was expressed from day 22, and another was from day 7. We then tested the effects of the miRNAs on vector removal by inserting one or both of the miRNA target sequences on either 3' UTR or 5' UTR of SeV L gene.

Upon induction of NSC differentiation, the cells infected with the vector containing both of the miRNA target sequences started to become vector-negative earlier than ones with only one of the miRNA target sequence. Further treatment with CPA increased the ratio of Nestin-positive cells from 85% to 99%, indicating that the undifferentiated cells harboring the vector can be efficiently removed. Taken together, the data indicate that appropriate combination of miRNA target sequences allows the auto-erasable system to select NSCs efficiently.

O-13

Pop2 is phosphorylated by Pho85 kinase to repress the expression of stress response gene *HSP12* upon glucose availability

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Pop2 protein of *S. cerevisiae* is a component of Ccr4-Not complex that is a conserved regulator of gene expression by shortening of poly(A) tail of mRNA. The Ccr4-Not complex may exert an additional control over transcription initiation by directly or indirectly inhibiting the function of the zinc finger transcription factor Msn2, which is known to control expression from the stress response element (STRE) in response to environmental signals. In this study, we found that Pop2 is phosphorylated at serine 39 residue (S39) in glucose medium. The dephosphorylation of S39 was occurred within 1 min of glucose depletion, and the addition of glucose to the glucose-deprived culture recovered this phosphorylation. In medium supplemented with glucose, Pop2 might be phosphorylated by Pho85 kinase at S39 to repress the expression of *HSP12*, encoding a small heat shock protein. In glucose starvation, Pho85 is inactivated and resulted in the derepression of *HSP12*, which is shown by the substantially elevated amount of *HSP12* mRNA in this condition. Our results suggest that Pop2 phosphorylation by Pho85 kinase is a part of glucose sensing system in yeast.

O-14

Confinement environment stress assessment via fNIRS: an update

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With astronauts (including Japanese astronauts) continuously recruited for long-term stays in the International Space Station (ISS), evaluation of the experienced confinement conditions is deemed compelling. Confinement in the same room with the same people is a possibly crucial psychosocial stressor for the astronauts. However, confinement-triggered psychological stress has yet to be efficiently assessed. Based on the proven negative effects of psychosocial stress on the frontal brain function, we evaluated stress-related indices of frontal brain function using functional near-infrared spectroscopy (fNIRS), a non-invasive brain-activity monitoring method measuring localized brain blood-oxygenation levels, additionally to conventional stress-evaluation methods, under confinement conditions.

The conditions experienced by astronauts were simulated using the Japan Aerospace Exploration Agency (JAXA) “confinement environment adaptation facilities”, where research participants were confined for 2 weeks. Confinement stress was evaluated by fNIRS measurements of frontal brain function during a cognitive test (verbal fluency test [VFT]), additionally to conventional stress evaluation methods, including the self-evaluation questionnaire of sense of coherence (SOC, 29-item scale). During confinement, exercise intervention was applied: control group (n=7, February 2016) had total exercise prohibition, intervention-group1 (n=8, December 2016) completed 15-minutes aero-bike training for 14 days, and intervention-group2 (n=8, December 2017) had a 5-day exercise prohibition, followed by 5 days of mandatory exercise, and again consecutive exercise prohibition.

Overall, fNIRS measurements generally tended to decrease, SOC scores had an increasing tendency, and interestingly, fNIRS measurements significantly differed between exercise intervention and control groups.

The fNIRS measurements’ general decrease suggests the deterioration of frontal brain function during confinement, indicating the possible detection of early signs of stress and depression using fNIRS. Despite the short confinement period, the SOC upward trend indicates that the experiences obtained under confinement strengthened the participants’ sense of coherence. Importantly, maintaining good frontal brain function during confinement environment stress can be assisted by exercise.

Ethical issues were reviewed and approved both from the University of Tsukuba Medical Ethics Committee (No.1022) and the JAXA Ethical Review Board. We declare no conflict of interest. This study was supported by the JSPS Grant in Aid for scientific research (15H05935).

O15(P-16)

Purification and Identification of Novel Tyrosinase Inhibitor from *Ganoderma formosanum*

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Ganoderma spp., commonly known as lingzhi or reishi, have been used as traditional Chinese medicine (TCM) for thousands years. Because of its multi-bioactivities and less toxicity, *Ganoderma* is a potential source for novel bioactivities discovery. In our previous study, ethyl acetate fraction of *G. formosanum* mycelium ethanolic extract (GFE-EA) exhibited inhibitory activity on tyrosinase, a key enzyme of melanogenesis. The aim of this study is to identify a novel tyrosinase inhibitor(s) from submerged cultivation of G. *formosanum*, an endemic species of Taiwan. GFE-EA was obtained by extracting lyophilized mycelia with 95% ethanol, concentrating and followed by liquid-liquid partitioning. Numerous column chromatography including silica gel 60, LH-20 and semi-preparative HPLC were set up. *In vitro* tyrosinase activity assay showed that GFE-EA 45, 50 and 55% sub-fractions from silica gel 60 chromatography exhibited 56.3%, 80.9%, 55.0% inhibitory rate. While 107 further fractionated fractions were obtained by LH-20 chromatography, eight of these partial purified fractions showed anti-melanogenic activity on tyrosinase-based TLC autography assay, a screening platform of tyrosinase inhibitors with naked eye. By comparing the HPLC profile, the major compounds of these 8 anti-melanogenic fractions had different retention time showing more than one tyrosinase inhibitor(s) might be isolated from GFE-EA. One major compound purified by semi-preparative HPLC with retention time 11.287 mins was targeted as a tyrosinase inhibitor. In following study, structural elucidations will be carried by high-resolution electrospray ionization mass spectrum (HRESI-MS) and nuclear magnetic resonance (NMR), as well as evaluation of potential mechanism using phenotype-based zebrafish model.

O-16

Phosphatidylserine exposure self-regulates mast cells' degranulation

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Mast cell (MC) degranulation significantly contributes to the pathogenesis of allergic response. CD300a is one of the inhibitory receptors expressed on MCs and suppresses IgE–mediated mast cell degranulation. Although CD300a was shown to be an important negative regulator in cytokine producing from mast cell upon recognition of phosphatidylserine (PS) exposed on the plasma membrane of apoptotic cells, its role in degranulation have not yet been investigated. Here we showed that CD300a inhibits degranulation of mast cells, which is independent of apoptotic cells. Live cell imaging indicated that PS was exposed quickly on live MC itself after antigen challenge and the exposed PS was co-localized with CD107a as well as CD300a. Furthermore, blocking CD300a-PS interaction by anti-CD300a antibody abolishes the inhibitory function of CD300a. Consistent with *in vitro* results, Cd300a deficient mice showed more body temperature decrease in a passive systemic anaphylaxis (PSA) model. Our study revealed an unidentified self-regulatory mechanism of MC degranulation via CD300a.

O-17

MafB is essential in Adipose Tissue Macrophages (ATM) for regulating thermogenesis.

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Introduction: Obesity and diabetes become an epidemic disease in modern times. Even though various treatment available for obesity & diabetes, there is a scarcity of durable treatment measures to cure obesity & diabetes in the long term. Recognition of active Brown Adipose Tissue (BAT) in Human (2009) suggests possibilities to curb obesity and Type-2 Diabetes (T2D) by increasing thermogenic metabolism of extra calories. In the implied model of cold-induced thermogenesis, the hypothalamic preoptic cold-sensitive neurons play a significant role by the sympathetic firing of catecholamine in BAT, which causes overexpression of thermogenic gene Ucp1 (Mitochondrial Uncoupling Protein-1) and increases the metabolic heat production. In the recent developments; Adipose Tissue Macrophages (ATM) role has been proven critical for innervation of these sympathetic neurons in the BAT. Furthermore, one of the recent studies indicates IL-4/IL-13 is crucial in ATM for cold-induced thermogenesis, and it is known that IL-4/IL-13 signalling can induce MafB expression, which is a bZip transcription factor important for myeloid cells differentiation and could have an essential role in ATM for regulating thermogenesis, need to be further scientifically explored.
Methods: We did Intermittent cold exposure at 8°C-8 hours alternate days for one week to either CL57BL/6J Wild-type mouse or CL57BL/6J Macrophage-specific *Mafb* deficient mouse (Mafb-Mϕ–CKO). On the day eighth we sacrificed the mice after 4-hour cold exposure and collected the BAT for RT-qPCR or Immunohistochemistry (IHC). We have used the following antibodies for IHC analysis:- Tyrosine hydroxylase (TH); which is a crucial enzyme for catecholamine synthesis and also used as a neuronal marker and Galectin 3/Mac2; a marker of macrophages cell lines.
Results: The RT-qPCR results of cold exposed wild-type mouse show an increase in *Mafb* and *Ucp-1* expression. The IHC staining with Mac2 & TH shows increase in macrophage numbers and sympathetic neuron innervations after cold exposure to the mouse. Furthermore, Measurement of cold exposed Mafb-Mϕ–CKO mouse body temperature by the rectal thermometer or using the MLX90614 sensor attached to the intracapsular region shows a decrease in body temperature with a comparison to the control mouse. Moreover, RT-qPCR analysis results Mafb-Mϕ–CKO cold exposed mouse show decrease in the *Mafb, Ucp-1* and *Th* gene expression level and IHC for Mac2 and TH shows a marked increase in macrophages number with a reduction in the tissue sympathetic neurons innervation with comparison to the control mouse.
Conclusion: Mafb-Mϕ–CKO mouse is deficient for adaptive thermogenesis and having increased tissue infiltration of macrophages with a decrease in tissue level of sympathetic neuron innervations.

O-18

Analysis on Transcriptional Regulation in The Early Phase of Somatic Cell Reprogramming

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Somatic cells can be reprogrammed into iPSCs by forcing express Yamanaka factors (Klf4, Oct4, Sox2 and c-Myc). iPSCs have great promise applications for drug screening, disease modeling, and regenerative medicine. However, reprogramming is inefficient and slow process, also their mechanism still remains incompletely understood. The improved idea on mechanism of this transformation may support to improve efficiency and accelerate reprogramming process. In the very early stage of reprogramming, beside morphology shifting (Mesenchymal to Epithelial Transtion – MET), apoptosis and senescence escaping, the depletion of somatic program is also required. Some published data shows the partially reprogrammed cells own incomplete repression of somatic related genes, and could be shifted to fully reprogrammed cells by knocking down these somatic genes. These results indicate that somatic related genes could be the roadblock inhibiting iPSC generation.

Many of somatic related genes are regulated by somatic selective transcription factors (SSTFs). In order to identify SSTFs that regulate somatic program and impair iPSC generation, we analyze gene expression between somatic cells and reprogramming cells at day 2 by Sendai virus vector-based reprogramming system. The advantage of the Sendai virus system is that they can reprogram somatic cells quite efficient compare to traditional retrovirus-based system. The screening identified six candidate SSTFs, overexpression one of SSTFs inhibited reprogramming by impairing MET. Research on epithelial to mesenchymal transition (EMT), a reverse process of MET which prevent iPSC generation, reveal key role of SSTF in triggering EMT. Together, our finding suggests that SSTF could resist pluripotent cell fate change and therefore suppression of the SSTF provide a potential strategy to enhance reprogramming efficiency.

O-19

Surveillance of Antimalarial Drugs Resistance Markers in Haiti for Best Practice toward Malaria Elimination

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BACKGROUND

Haiti, located in the Caribbean Hispaniola island is a special case where malaria is mainly caused by *Plasmodium falciparum* (Pf) and despite chloroquine (CQ) being introduced since 1955, it is still the main drug in use for treatment of uncomplicated malaria.

This practice started to be questioned after the first publication about the presence of CQ-resistant haplotype in Haiti in 2009. We aimed at genotyping a CQ resistance marker, Pf chloroquine resistance transporter (pfcrt) and an artemisinin resistance marker, kelch 13 (k13) in Pf isolates from Haiti to continue molecular surveillance and provide recommendation for clinical practice in the wake of malaria elimination.

METHODOLOGY

Febrile patients were recruited in 3 departments of southern Haiti from August to September 2017. Pf positive samples were first selected by the Loop-Mediated Isothermal Amplification method as potentially eligible. Those confirmed by a nested-PCR targeting the 18s rRNA gene, were finally included for pfcrt and k13 analysis.

Using DNA extracted from dried blood spots, a segment including codons 72~76 of pfcrt and the propeller domain of k13 were amplified by nested-PCR followed by direct sequencing of secondary PCR products.

RESULTS

Seventy-eight (78) samples were finally included. All analyzed samples presented the wild type amino acid sequence CVMNK at positions 72~76 of pfcrt. None presented any resistance-associated polymorphism of k13. Five samples presented a synonymous mutation at nucleotide 1359 (bp: T1359A, codon: G453) of k13.

CONCLUSION

No drug resistance-associated mutation was detected for pfcrt and k13 in samples from highest transmission areas of Haiti. Indeed, the reported scant CQ-resistant haplotypes aren't confirmed autochthonous. We can assert that CQ-resistant and artemisinin-resistant haplotypes are not consistently circulating in Haiti.

CQ still remains the treatment of choice for uncomplicated *Plasmodium* infection while achieving the target malaria elimination in Haiti. Artemisinin-based therapy can be an alternative for extraordinary cases.

O-20

Sorafenib-loaded Silica-containing Redox Nanoparticle - an Orally Administered Drug nanocarrier for Effective Treatment of Liver Fibrosis

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Liver fibrosis is the results from long-term liver injuries lead to cirrhosis, liver failure and liver cancer. The major effector cells that regulate hepatic fibrotic progression are hepatic stellate cells (HSCs). Therefore, inactivation of hepatic stellate cells (HSCs) is a key approach for inhibition of hepatic fibrotic. Sorafenib is a tyrosine kinase inhibitor possessing potential anti-fibrotic activity to inactivate HSCs. However, sorafenib has a low bioavailability due to its poor solubility and its adverse effects which are causing by long-term using drug with high dose drug administration. Recently we have been developing silica-containing redox nanoparticle (siRNP), which possesses crosslinking structure by silica moieties improving absorption of hydrophobic drugs in addition to antioxidant properties. The objective of this research is to investigate the therapeutic effect of sorafenib-loaded siRNP (sorafenib@siRNP) to treat liver fibrosis via oral administration route. Sorafenib@siRNP was simply prepared by self-assembling the mixture of sorafenib and an amphiphilic redox polymer in an aqueous phase in the presence of TEOS (tetraethoxysilane). siRNP exhibited high drug loading capacity due to the adsorption character of the silica in the nanoparticle and suppressed possible adverse effects of sorafenib in gastrointestinal tract due to its ROS scavenging activity. As compared to free sorafenib, the treatment of sorafenib@siRNP significantly inhibited the proliferation of HSCs *in vitro* but it presented low toxicity to normal endothelial cells. Oral administration of sorafenib@siRNP to mice improved significantly blood uptake of sorafenib in plasma and liver compared to free sorafenib, although siRNP did not internalized to blood stream. Therapeutic efficacy of sorafenib@siRNP was evaluated in CCl_4-induced liver fibrosis model of mouse and the results showed that orally administered sorafenib@siRNP exhibited the highly protective and therapeutic effects on liver fibrosis without any noticeable adverse events. These results suggest that sorafenib@siRNP is a promising oral nanomedicine for treatment of liver fibrosis.

O-30

Development of molecular target therapy for human acute GVHD in humanized mice

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Acute Graft-versus-host-disease (GVHD) is a life-threatening complication following allogeneic hematopoietic stem cell transplantation. It is known that the transferred $CD4^+$ T cells and $CD8^+$ T cells recognize alloantigens presented by recipient cells and cause the tissue injury. However, the effective molecular target therapy for human acute GVHD has not been established.

DNAX accessory molecule-1 (DNAM-1) is expressed on immune cells including T cells and induces an activating signal upon binding its ligands CD155 and CD112 expressed on almost all of the nucleated cells. It was reported that DNAM-1-mediated signal induced Th1 response and cytotoxicity. Moreover, we previously demonstrated that DNAM-1 contributed to the pathogenesis of acute GVHD, and the administration of anti-mouse DNAM-1 monoclonal antibodies (mAb) dramatically improved acute GVHD in a mouse model. However, it remains to be clarified whether DNAM-1 is involved in human acute GVHD and whether anti-human DNAM-1 mAb is also effective for human acute GVHD.

To address this issue, we generated human CD155-expressing NOG transgenic mice (hCD155-Tg NOG mice). By using these mice, we established the human acute GVHD model, in which hCD155-Tg NOG mice were transferred with human peripheral blood cells. To examine whether human DNAM-1 is involved in the pathogenesis of acute GVHD, we injected anti-human DNAM-1 neutralizing mAb intravenously into the human acute GVHD mice model. Treatment with anti-human DNAM-1 mAb in a prophylactic setting significantly prolonged survival, indicating that human DNAM-1 was also involved in the pathogenesis of human acute GVHD. Moreover, the mice treated with the anti-human DNAM-1 mAb in a therapeutic setting also exhibited significantly prolonged survival. These results suggest that the anti-human DNAM-1 neutralizing mAb can be an effective therapeutic strategy for acute GVHD in human.

O-22

H_2O_2 is involved in K_{ATP} channel dysfunction induced by TP receptors activation in rat aorta

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Introduction: Hydrogen peroxide (H_2O_2) suppresses ATP-sensitive potassium (K_{ATP}) channel activity in vascular smooth muscle cells (VSMC). Activation of thromboxane A_2 (TP) receptors stimulates reactive oxygen species (ROS) production and decreases K_{ATP} channel activity in VSMC. This study aimed to investigate whether endogenous production of ROS by TP receptors activation reduces relaxation mediated by K_{ATP} channel.
Methods: Concentration-effect curves to the K_{ATP} channel activator Pinacidil were performed in the presence of vehicle, a superoxide anion scavenger (Tiron) or Catalase, which decomposes H_2O_2, in rat aortas contracted with the TP receptors agonist U46619. ROS production induced by U46619 was measured in endothelial cells and VSMC by flow cytometry. Results are expressed as the mean ± SEM. The level of statistical significance was defined as $p<0.05$.
Results: The K_{ATP} channel activator Pinacidil induced aorta relaxation and its effect was dependent on the concentration of U46619 used to stimulate contraction: 10 nM: 98.7 ± 2.2%; 0.1 μM: 72.0 ± 1.4%; 1 μM: 50.7 ± 3.5%, n= 5-6. U46619-induced contractions were similar at the concentrations of 0.1 μM (3.0 ± 0.1 g, n=6) and 1 μM (3.3 ± 0.2 g, n=5), but lower with 10 nM (1.3 ± 0.1 g, n=5). In aortas contracted with 0.1 μM U46619, the relaxation induced by Pinacidil was potentiated by Catalase (pD_2: Pinacidil+vehicle: 6.01 ± 0.08, n=5; Pinacidil+Catalase: 6.32 ± 0.06, n=5), whereas Tiron had no effects. U46619 increased ROS production in endothelial cells [(units of fluorescence, UF) Control: 11.2 ± 0.8, n=5; U46619+vehicle: 13.7 ± 0.2, n=5)], an effect abolished by the TP receptors antagonist SQ 295448 (SQ, 10 μM) (11.8 ± 0.3, n=5). Tiron and PEG-Catalase decreased ROS production induced by U46619 (U46619+vehicle: 32.6 ± 1.2, n=5; U46619+Tiron: 28.3 ± 0.3, n=5; U46619+PEG-Catalase: 24.6 ± 0.8, n=5). U46619 also increased ROS in VSMC (Control: 7.9 ± 0.1, n=5; U46619+vehicle: 9.6 ± 0.1, n=5) and this was abolished by SQ (U46619+SQ: 8.0 ± 0.1, n=5). Tiron and PEG-Catalase reduced ROS production induced by TP receptors activation in VSMC (U46619+vehicle: 5.0 ± 0.0, n=5; U46619+Tiron: 4.2 ± 0.1, n=5; U46619+PEG-Catalase: 4.2 ± 0.0, n=5).
Conclusion: H_2O_2 produced by TP receptors activation decreases relaxation mediated by K_{ATP} channel activation, further supporting a key role for ROS in the modulation of vascular tone.
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O-23

Acting out of dreams: Neural mechanisms of REM-sleep behavior disorder and its association with synucleinopathy

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Over the night, we repeatedly cycle between non-rapid eye movement (non-REM) sleep, REM sleep, and short arousals. During REM sleep, our brain cortex becomes activated and produces vivid dreams. Yet, we usually do not act out of our dreams owing to expression of muscle atonia, a major physiological characteristic of REM sleep. However, patients with REM sleep behavior disorder (RBD) exhibit impaired muscle atonia during REM sleep and frequently act out of their dreams, which is likely due to local damage in the brain. RBD patients suffer from excess daytime sleepiness and fatigue due to dream-related aggressive behaviors during nighttime, and currently no effective treatment exists for severe RBD.

Importantly, there are reports that more than 90% of patients with RBD eventually develop synucleinopathies such as Lewy body dementia and Parkinson's disease within 15 years (Iranzo et al., Lancet Neurol. 12:443, 2013). Synucleinopathy is a neurodegenerative disease caused by abnormal accumulation of α-synuclein protein in the brain and results in various symptoms including movement disorders and cognitive impairment. Understanding the mechanisms of RBD and applying it to future clinical studies is crucial both for improving the quality of patients' life and for therapeutic intervention before progression of synucleinopathies. However, the neural mechanisms underlying association of RBD with synucleinopathy remain elusive, and no current rodent model of synucleinopathy exhibits RBD.

Here, to elucidate the mechanisms of RBD and its association with synucleinopathy, we developed genetic tools that allow local expression of pathogenic forms of α-synuclein in restricted brain areas in mice. Then we applied it to various brain regions that express high levels of endogenous α-synuclein. As a result, we succeeded in establishing a mouse model with RBD-like phenotype. Our mouse model is expected to provide important clues about how RBD develops at early stages of synucleinopathy and also provide a platform for developing treatments for RBD.

O-24

Dead Bifidobacterium longum induced the anti-aging effect to *C. elegans*

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We have microbes in our intestine. These microbes form our intestinal flora and are called Probiotics. They are beneficial for our health. *Bifidobacterium longum* (BL) is a typical Probiotics and generally included in yoghurt and supplemental foods. It is one of the most famous microbes. So far, a wide variety of physiological effects of BL have been reported, however, the physiological effect for longevity and anti-aging was seldom known. The aim of this study is to analyze the physiological effects of BL for stress tolerance, anti-aging and longevity in *C. elegans*. In this study we used the dead BL (BR-108). When we eat BL, almost all BL will die by gastric acid or some other digestive enzymes. To be more practical we adopted dead microbes.

C. elegans is a kind of worms and it is easy to culture. It has many kinds of orthologue genes with higher animals. Hence it is widely used as a model organism. In general *C. elegans* eat *E. coli* as food. Therefore we fed them on BL with E. coli.

As a result, dead BL prolonged the lifespan of *C. elegans*. In general the movement of *C. elegans* decreases age-dependently. However worms fed on BL kept the movement. Furthermore BL increased several kinds of stress tolerance of worms. The result of qPCR indicated dead BL upregulated the transcription of several genes in worms related to stress tolerance. In this study we elucidated these physiological effects were induced through several genes in IIS pathway and p38 MAPK pathway. These pathways are known to relate to longevity, anti-aging and stress tolerance. Therefore dead BL possibly induced anti-aging effects to *C. elegans* through these pathways.

O-25

Roles of the interaction between THG-1 and NRBP1 in the development of esophageal squamous cell carcinoma

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Esophageal cancer is the sixth leading cause of death by cancer, and eighth most frequent form of cancer in the world. Adenocarcinoma and squamous cell carcinoma (SCC) are the major histological types of esophageal cancer. Esophageal squamous cell carcinoma is the most common type of esophageal cancer in Asian countries. It occurs in stratified squamous epithelium of the esophagus which has several layers of epithelium. Despite the development of anti-cancer therapy, Esophageal SCC has shown a high level of robustness against environmental stresses, metabolic disorders and therapeutic efforts.

We have identified a novel molecular mechanism of the SCC development by THG-1, a Tsc-22 family protein. THG-1 is localized in the basal layer of normal squamous epithelium and ubiquitously expressed in SCC. THG-1 knockdown in esophageal SCC cells suppresses the cell proliferation, invasiveness and tumorigenesis. To discover the molecular mechanism of SCC development mediated by THG-1, we investigated the THG-1 interacting molecules by proteomics approach. We found that THG-1 interacts with several molecules that regulate proliferation, metabolism and response to the microenvironmental factors. Among THG-1 interacting proteins, we focused on Nuclear receptor binding protein 1 (NRBP1). NRBP1 was first identified as a multidomain putative adaptor protein, containing two putative nuclear receptor binding motifs (LXXLL), a putative binding domain for Src homology-2 (SH2) domain, a pseudo-kinase domain and a bipartite nuclear localization signal. NRBP1 has been identified as a Cullin5-type E3 ubiquitin ligase, and recent studies implied that NRBP1 has tumor suppressive functions. Expression of NRBP1 was downregulated in lung and colorectal adenocarcinomas, and its low expression correlates with poor prognosis and survival rate. In addition, it was confirmed that NRBP1 negatively regulates Ras signaling pathway and β-catenin/WNT signaling pathway. This context suggests that NRBP1 is an important tumor suppressor *in vitro* and *in vivo*.

We therefore investigated the function interaction between THG-1 and NRBP1 to find the molecular mechanism how THG-1 overexpression functions in the development of esophageal squamous cell carcinoma.

O-26

MAIR-II deficiency suppresses M1/M2 polarization protecting against cardiac dysfunction post-myocardial infarction

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Background: MAIR-II (Myeloid-Associated Immunoglobulin-Like Receptor II) is expressed on myeloid cells and involved in Toll-like receptor-4 (TLR4)-mediated inflammatory monocyte migration from the blood to sites of infection as part of the host response in polymicrobial peritonitis. In myocardial infarction (MI), monocytes are known to be recruited to infarcted myocardium followed by a response of inflammatory M1 and anti-inflammatory M2 macrophages. However the role of MAIR-II in MI remains elusive.

Purpose: To determine the role of MAIR-II in MI pathophysiology.

Methods and Results: Flow cytometric analysis revealed that MAIR-II^{+} myeloid cells were abundant from post-MI days 3 to 5 in infarcted hearts induced by permanent ligation of the left coronary artery. To address MAIR-II's role in myeloid cell function *in vivo*, effects from MAIR-II deficiency were investigated. In echocardiography, MAIR-II knockout (KO) mice had thicker left ventricle posterior walls and higher ejection fractions compared to wild-type (WT) mice. This indicates that MAIR-II deficiency leads to favorable post-MI remodeling. After further investigation, we found that MAIR-II KO hearts had less macrophage influx and more neutrophil infiltration after MI. Moreover, In MAIR-II KO there was less Il1b inflammatory gene expression, less Tgfb and collagen type I alpha 2 fibrotic gene expressions, and more CD206^{+} M2 macrophages in infarcted hearts compared to WT. To elucidate MAIR-II's role in macrophages, we analyzed bone marrow-derived macrophages (BMDM) from WT and MAIR-II KO mice polarized to M1 and M2 using LPS or IL-4 respectively. We found that M1 and M2 polarized BMDM from MAIR-II KO expressed less M1 and M2-related gene expressions compared to WT mice. Furthermore, in M1 polarized MAIR-II KO BMDM, attenuated inflammation in activated monocytes expressed by NLRP-12 was higher than WT.

Conclusion: MAIR-II is involved in MI pathophysiology, by exacerbating inflammation and fibrosis through M1/M2 polarization. A deficiency in MAIR-II shows promising therapeutic effects against cardiac dysfunction post-MI.

O-27

Adenosinergic Mechanisms of Sleep Control in the Nucleus Accumbens

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We have revealed a prominent role of adenosine A_{2A} receptor (A_{2A}R)-expressing neurons in the nucleus accumbens (NAc) in sleep/wake regulation and proposed a novel brain circuit for sleep control by motivated behavior (Oishi Y., et al., Nat. Commun., 8:article 734, 2017). This brain circuit may explain the tendency to fall asleep in the absence of motivating stimuli, i.e., when bored. We hypothesized that the ability of the NAc to induce sleep is mediated by the classic somnogen adenosine. Adenosine can be formed by various processes in all types of cells but the neuro-dynamics of adenosine (release, receptor activation, etc.) in controlling sleep are still unclear. In this study, we ablated glial fibrillary acidic protein (GFAP)-positive cells in the NAc of mice by virus-mediated expression of diphtheria toxin (DT) receptors and intraperitoneal administration of DT. After analyzing electroencephalogram and electromyogram recordings of the mice, we found a remarkable increase in slow-wave sleep (SWS) one week after DT treatment that was, surprisingly, accompanied by an increased number of GFAP cells in the NAc. *In-vivo* microdialysis one week after DT treatment revealed a significant increase of extracellular adenosine in the NAc. Moreover, sleep/wake behavior was unchanged after ablation of GFAP cells in the NAc of A_{2A}R knockout mice. Our current results may suggest that adenosine from NAc glial cells plays an important role for sleep control.

O-28

Effects of Chronic Stress on Sleep in Mice

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Depression is a mental disorder characterized by loss of interest or pleasure and increase in anxiety and hopeless feelings. In addition to these psychiatric symptoms, patients also suffer from physical symptoms such as sleep disorders and increase or decrease in body weight. In particular, sleep disorders are observed in most of the patients. Sleep consists of two stages: REM (rapid eye movement) sleep and non-REM sleep. In patients with depression, abnormalities in REM sleep are frequently observed. For example, the REM sleep latency (the time taken to enter the first REM sleep cycle) is shortened and the duration of the first REM sleep episode is prolonged. Related to these changes in REM sleep, there are controversies as to whether REM sleep plays an important role in the recovery from depression or REM sleep rather contributes to worsening of the depression-related symptoms. As a first step to elucidating the causal relations between sleep abnormalities and depression using genetics, we aimed to clarify how sleep is affected in a mouse model of depression induced by chronic stress. As a stressor, water immersion and restraint stress was used. This method leads to various depression-like phenotypes in mice (Mizoguchi K et al., *J. Neurosci.*, 2000, 20(4):1568-1574; Miyata S et al., 2011, *PLoS One* 6(5):e19859). Consistent with previous reports, mice exposed to this chronic stress exhibited despair- and anhedonia-like behavioral phenotypes and reduction in body weight. When sleep was measured, there were large changes in various measures including REM sleep amounts. Based on these results, we expect that the mouse model of depression will provide a valuable platform for solving the conflicting argument about the relations between REM sleep and depression.

O-29

Primitive hematopoiesis does not require LSD1 demethylation activity in zebrafish

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Primitive hematopoiesis is embryonic-type hematopoiesis that produces only erythrocytes and macrophages independently from hematopoietic stem cells. We previously isolated zebrafish LSD1 mutant that shows defects in primitive hematopoiesis. LSD1 (lysine-specific histone demethylase 1) is an enzyme that epigenetically regulates the expression of various genes via demethylation of histone H3 K4 or K9. The demethylation activity of LSD1 has been reported to be important for a variety of *in vivo* LSD1 functions, such as differentiation of ES cells and brown adipocytes, and hypoxia response. While, there are also reports that showed it is not required for other LSD1 functions, for example, maintaining circadian clock homoeostasis and stabilizing the estrogen-related receptor. Based on these facts, we wondered whether primitive hematopoiesis requires demethylation activity of LSD1 or not.

Here we performed sets of phenotypic-rescue experiments of zebrafish LSD1 mutants by overexpressing wild-type or enzymatically-inactive LSD1. A lysine residue at the position 661 in human LSD1 is a catalytic center of the demethylation activity, and a point mutation (K661A) completely abolished this activity. Since the amino acid residue corresponding to Lysine 661 in human LSD1 is Lysine 638 in zebrafish LSD1, we constructed K638A as an enzymatically-inactive zebrafish LSD1. Overexpression of both wild-type and K638A zebrafish LSD1 rescued the expression of erythroid marker *gata1*. We next analyzed the phenotypic-rescue activities of other zebrafish mutant LSD1 with point mutations at important positions for its enzymatic activity (N512A、D533A、F537A). Overexpression of all these mutant LSD1 also rescued the *gata1* expression. Finally, we carried out phenotypic-rescue experiments using human LSD1 and its enzymatically inactive mutant K661A. As a result, overexpression of both wild-type and K661A LSD1 rescued the expression of *gata1* in zebrafish LSD1 mutants.

All of these data suggest that primitive hematopoiesis does not require LSD1 demethylation activity in zebrafish

O-21

Glucocorticoid-impaired Wound Healing Ability of Mesenchymal Stem Cells and Endothelial Progenitor Cells by the Distinct Defect of SDF-1/CXCR4 Cascade

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Glucocorticoids (GCs) are known as the most effective anti-inflammatory agents, however, their application is accompanied by severe side effects, including impairment of wound healing. Wound healing is a complex process that requires the contribution of various cell types. Central to this, mesenchymal stem cells (MSCs) and endothelial progenitor cells (EPCs) play crucial roles. While MSCs contributes to the process mostly via the paracrine effects to recruit numerous of cells to the wound sites, EPCs involves in the neovascularization. Up to now, the influences of GCs on the wound healing ability of MSCs and EPCs have not yet been clarified.

In the present study, we found that GCs impaired the wound healing capacity of Adipose Tissue-derived MSCs (AT-MSCs) and EPCs, via distinct interference on wound healing gene expression in a cell type-dependent manner. Expression of stromal cell-derived factor 1 (SDF-1) was diminished in AT-MSCs in the presence of GCs but not in EPCs. On the contrary, the expression of SDF-1 receptor, CXCR4 was diminished in EPCs in the presence of GCs but not in AT-MSCs. Of note, we found that impairment of prostaglandin (PGE2) production by the reduction of PGE2 synthases related gene expression, COX2 and mPGES1, was responsible for the reduced expression of SDF-1 and CXCR4, respectively. Importantly in EPCs, GCs impaired the expression of prostaglandin receptor EP4. Treatment with PGE2 in the presence of GCs, recovers the CXCR4 expression and wound healing ability, through upregulation of EP4 expression and activation of the PI3K/AKT pathway. Furthermore, under hypoxic conditions, GCs downregulated the expression of CXCR4 via the impairment of HIF2α pathway in a different manner.

Our findings highlighted the negative effects of GCs on EPCs and MSCs function, suggesting that autologous progenitor cell transplantation derived from patients who have received GCs treatment for a long time should be carefully considered.

O-31(P-11)

Identification and characterization of potential partners of BILBO1 in the parasite *Trypanosoma brucei*

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Trypanosoma brucei is a protozoan flagellate parasite responsible for Human African Trypanosomiasis commonly known as sleeping sickness and Animal African Trypanosomiasis, or Nagana, in livestock. Diagnosis and treatment of trypanosomiasis remain complex and need to be improved for eventual elimination. This parasite has several organelles such as a single mitochondrion, a motile flagellum nucleated from basal bodies and a specialized compartment called the flagellar pocket (FP). The FP is an invagination of the plasma membrane and the only site for endo- and exocytosis. A cytoskeletal structure called the flagellar pocket collar (FPC) is localized at the neck of the FP, the exit area for the flagellum outside the cell. In 2008, our group identified and characterized the first component of the FPC, a cytoskeletal and scaffold protein called BILBO1[(1)] . Previous work has shown the essential role of BILBO1 in FP and FPC biogenesis, endocytic activity, repositioning of the new flagellum and cell viability[(2)]. Recently, we identified the first protein partner of BILBO1 localized at the FPC named FPC4 [(3)]. To identify new putative partners of BILBO1, a Yeast-Two-Hybrid (Y2H) screen has been carried out. In this study, we describe an experimental approach for identification and functional characterization of those new partners by performing *in situ* gene tagging and knockdown experiments by RNA interference. Our preliminary data show that these identified proteins localize to different regions of the FP, in close proximity to BILBO1 and, with further investigation, may have promising distinct roles in the biogenesis of the flagellar pocket.

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O-32

Resident memory CD8 T cells with a potential of producing IL-17A are already distributed in disease-naïve nonlesional sites of psoriasis

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Psoriasis is a chronic inflammatory skin disease. Although various descriptions revealed a role of IL-17A producing CD4 T cells in psoriasis pathogenesis, there have been many reports regarding the dominance of CD8 T cells, especially in epidermis, in psoriasis and skin CD8 T cells are reported to be required for lesion formation of psoriasis. However, the phenotypical characteristics of these CD8 T cells have been obscured. Recent studies have confirmed that human skin is populated by non-recirculating resident memory T cells (T_{RM}s). In order to investigate the relation of CD8 T_{RM}s in psoriasis disease formation, T cells were isolated from lesional, disease-naïve nonlesional sites of plaque-type psoriasis patients (n=17) and from normal skin specimens after surgery (n=15), and profiles of T_{RM} were compared among three groups. Skin specimens were also processed for immunohistological evaluation. Then both lesional and nonlesional sites of psoriasis, especially epidermis, were dominated with $CD69^+CD103^+$ CD8 T_{RM}s. This population even from nonlesional sites, which have never experienced disease formation, showed stronger potential of producing IL-17A, compared to normal skin. These results demonstrate that $CD69^+CD103^+$ CD8 T_{RM} are already distributed in disease-naïve nonlesional skin holding IL-17A-producing phenotype and possibly contributes to “ready-to-go condition” for disease formation.

O-33(P-4)

Unveiling of Extracellular miRNA Profiles of Breast Cancer

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In an era of precision medicine, biomarker discovery is indispensable for novel therapeutics to optimize treatment efficacy. MicroRNAs within patient serum have emerged as novel diagnostic biomarkers for several diseases. They are essential regulators of global mRNA expression in cells. Aberrant regulation of miRNA can result in tumor initiation, drug resistance and metastasis in cancer. miRNA assays are convenient for large-scale studies covering multiple miRNA targets and realistic in screening across diverse breast cancer types for early detection or factors that drive cancer progression. In this study, we collected patient serum samples from 4 major molecular subtypes: luminal A, luminal B, triple negative and HER2 type, and breast cancer patients with benign tumor and ductal carcinoma in situ (DCIS). Microarray analysis of miRNA expression was utilized and unique serum miRNA signatures between non-cancer and breast cancer cancer patients were identified. While early diagnosis aids in effective management of breast cancer, prognosis is also important to patients during the course of treatment. Thusly, we observed specific miRNA profiles across breast cancer subtypes, suggesting that secreted miRNA coincide with the secreting cancer cell. Moreover, specific clusters of miRNAs demonstrated changes in expression levels over the course of time and varies across subtypes. These trend differences suggest diverse roles taken up by the cancer cell during specific time-points of cancer progression. Through classifying these heterogeneous compositions of the cancer cell, molecular mechanisms underlying these identified biomarkers can be essential in developing effective treatments and translational research is needed.

O-34

Protective Effect of Persulfide to Block Peroxidation of Protein Cysteine Residue during Oxidative Stress

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We found that persulfide species (R-SSH) such as cysteine persulfide, glutathione persulfide, and protein-bound persulfide, produced *in vivo* by CSE and CARS2, have been proposed as higher nucleophilic and powerful reductants than mono-thiols to scavenge reactive oxygen species (ROS) (Ida et al., *PNAS* 2014; Abiko et al., *Chem Res Toxicol* 2015; Akaike et al., *Nat Commun* 2017). However, functions of persulfide on proteins are still unclear. We speculated that they might act as cellular protectants and redox signaling intermediates during exposure to oxidants. To address this, we have focused on protein tyrosine phosphatase (PTP) 1B, which is a negative regulator of EGFR, because PTP1B as a well-known sensor protein with reactive cysteine that easily undergoes *S*-oxidation by ROS and/or *S*-sulfhydration (SSH conversion) by persulfides.

Incubation of PTP1B with sodium disulfide (Na_2S_2) converted its SH of Cys215 in the active site to SSH and inhibited the enzyme activity. This inhibited activity was restored by dithiothreitol (DTT) through reduction of the SSH to SH. Although excessive ROS causes irreversible oxidation of SH to SO_3H, we postulated that reaction products of SSH with ROS (SSOH, SSO_2H, and SSO_3H) could be reduced by reductants. Consistent with this, we detected SSOH at the Cys215 as a result of a reaction of PTP1B with H_2O_2 in the presence of Na_2S_2. Interestingly, SSOH was reduced to SH by DTT and the inhibited PTP1B activity was restored. Under these conditions, oxidized PTP1B (SO_3H) detected by Western blotting was decreased in the presence of Na_2S_2, suggesting a reversibility of SSO_nH (n = 1~3) associated with recovery of PTP1B activity. When S-S bond of PTP1B-SSOH was cut by DTT, the SOH should be released from the protein. To confirm this, we labeled the SSOH with dimedone to form SS-dimedone and incubated the labeled protein with DTT. As expected, the S-S bond was cleaved by DTT, resulting in releasing of 2-thiodimedone from PTP1B-SS-dimedone.

Taken together, the present study indicates, for the first time, that *S*-sulfhydration at Cys215 of PTP1B by persulfides appears to protect the active site against irreversible *S*-oxidation by excessive ROS. These suggest that persulfide species play a role in conservation of reversibility of *S*-modifications of sensor proteins to maintain redox signaling.

O-35

The Effects of Siblings of children with severe motor and intellectual disabilities on Family Empowerment Factors

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[Background & Aim]

Siblings of children with sever motor and intellectual disabilities (SMID) have a role of sympathetic supporters for their family members and of building good relationships with them. However, the previous study also reported that parents require more social resources for caring for siblings. In our study, we found that common and different factors related to family empowerment of both families raising SMID children with/without siblings in order to support them effectively.

[Methods]

We conducted the questionnaire survey for family members raising a child with severe motor and intellectual disabilities by mail to 212 special schools for physically handicapped children in all prefectures in Japan. The questionnaires were composed of Family Empowerment Scale Japanese version (J-FES), and their family attributions and health related outcomes. Multiple regression analysis was conducted for revealing the associated factors with family empowerment; J-FES scores were objective variables. The significance level was set at 5 %.

[Results]

Regardless of with/without siblings of SMID children, the common factors contributed to family empowerment were the following ones; frequent use of social resources, lots of support organizations, higher household income, and less burden of care. Families with siblings of SMID child had higher family empowerment with longer time of visiting service. Families without siblings showed higher family empowerment with frequent relationship with the most reliable organization and older age of primary caregivers.

[Conclusions]

This study presented that the common factors related to family empowerment of families with/without siblings of SMID children. On the other hand, there were different factors on family empowerment on only one group. We should consider these results and support their family empowerment with respect to not only other family members but also siblings of SMID children.